is not necessary, or protein production is carried out as a basic study.

Page 15, please amend the first paragraph as follows:

After translation, an active protein may be obtained. Even when the resultant protein is not an active protein, it may be converted to an active protein by a variety of techniques. In many cases, a protein is first synthesized at the ribosomes in the cytoplasm as an inactive precursor (pro-form) which comprises an active protein bearing at the N-terminus thereof a peptide of about 15 to 60 amino acids responsible for secretion (secretory signal). The peptide region, which functions as a secretory signal, is concerned with the mechanism of passing through the cell membrane, and is removed by cleavage with a specific protease during the passage through the membrane (not always) to yield a mature protein. The peptide moiety which functions as a secretory signal has a broad hydrophobic region comprising hydrophobic amino acids in the middle of the sequence, and basic amino acid residues at a site close to the N-terminus. A secretory signal may be understood as a synonym of a signal peptide.

Page 15, please amend the second paragraph as follows:

In addition, in some proteins, a peptide moiety which functions as a secretory signal is further attached to the N-terminus of an inactive precursor (pro-form), and such a protein is called as a prepro-protein (the prepro-form). For example, trypsin is present as a prepro-form immediately after translation into amino acids, as a pro-form after being secreted from cells, and is converted into active trypsin in the duodenum upon limited degradation by enteropeptidase or by self degradation. A pro-form from which an active protein region has been deleted is called a pro-region, a prepro-form from which a pro-form region has been deleted is called a pre-region, and a prepro-form from which an active protein region has been deleted is called a prepro-region.

Page 16, please amend the first paragraph as follows:

The "secretory signal nucleotide sequence", which is one of the essential components of the protein expression vector of the present invention, refers to the nucleotide sequence encoding a secretory signal. Also, the "secretory signal" refers to the pro-region when a protein is expressed as a pro-form, and at least the pre-region or the prepro-region when a protein is expressed as a prepro-form. However, the secretory signal is not limited in so far as it is capable of secreting the intracellularly expressed protein,

extracellularly. The secretory signal nucleotide sequence constructed within the protein expression vector of the present invention preferably encodes a secretory signal with a cleavage site at the C-terminus of the signal. When the sequence encodes a secretory signal that does not contain a cleavage site at the C-terminus, it is preferred to newly insert a nucleotide sequence encoding a cleavable site at the 3' end of said secretory signal nucleotide sequence. This is, for example, a trypsin signal represented by 1st to 23rd amino acids in SEQ ID NO: 19. At the C-terminus (19th to 23rd amino acids) of said sequence, there is Asp-Asp-Asp-Asp-Lys which is recognizable by enterokinase.

Page 17, please amend the first paragraph as follows:

Since the secretory signals of eukaryotic cells are similar to those of prokaryotic cells, *Escherichia coli* and the like may be used as the host. Since the secretory signal has different extracellular secretory activities depending on the host, it is necessary to select a secretory signal appropriate to the host. Specific examples of secretory signals include IgG (κ) (or IgGk) signal (or leader) and trypsin signal, which exhibit particularly high secretory activities when insect cells or mammalian cells are used as the host cells. Other examples of secretory signals include

BiP of flies (*Drosophila*), melitin of honeybees, α-factor of Pichia pastoris, PHO, and the like. When a trypsin signal is referred to herein, it may be constructed by either the 1st to 18th amino acids or the 1st to 23rd amino acids in SEQ ID NO: 19. Further, the secretory signal also includes, other than those exemplified above, their homologs and variants which are capable of secreting proteins extracellularly.

Page 17, please amend the second paragraph as follows:

The "Tag nucleotide sequence", which is another essential component of the protein expression vector of the present invention, refers to a nucleotide sequence that encodes a Tag sequence. The "Tag sequence" refers to an amino acid sequence that is not derived from the nucleic acid encoding a target protein and is inserted in order to facilitate, when expressed, isolation, purification and recognition of the target protein. Therefore, such a Tag sequence may be, for example, an antigen or an epitope recognizable by an antibody. By retaining the recombinant fusion protein containing a Tag sequence in a substance capable of recognizing said Tag sequence, isolation and purification can be carried out easily.

Page 24, please amend the first paragraph as

follows:

Introduction of the above expression vectors into the host cells per se may be conducted by employing a conventional method which includes, for example, transfection by the lipopolyamine method, the DEAE-dextran method, Hanahan's method, the lipofectin method, the calcium phosphate method, microinjection, electroporation, and the like.

Page 26, please amend the second paragraph as follows:

Plasmid pSecTag2A (1 μg, 0.1 μl) was treated with the restriction enzymes Nhe I and BamH I to completely remove the region encoding IgGk leader sequence. To this solution were added 100 pmoles each of the sense DNA and the antisense DNA described above, and the mixture was treated at 70°C for 10 minutes, after which it was left standing at room temperature for 30 minutes to allow annealing. To 1 μl each of the His secretory signal sequence, which had been treated with Nhe I and BamH I, and pSecTag2A was added 2.0 μl of solution I of DNA Ligation Kit Ver. 2 (Takara Shuzo Co., Ltd.), and the mixture was allowed to react at 16°C for 30 minutes. To the reaction mixture was added 0.1 ml of competent *Escherichia coli* cells XL1-Blue (Stratagene Company), and the mixture was allowed to react on ice for 30 minutes, followed by heat shock